REMARKS

Applicant thanks Examiner Gabel for the courtesies extended to Applicant's representative during the telephonic interview conducted on March 11, 2003.

Claims 2-13 and 15-20 are currently pending in the application. In order to advance prosecution, Applicant has canceled claims 15, 17, and 18, amended claims 2, 4, 6-8, 11, 13, 16, and 19-20, and added claim 21-23. A complete listing of all the claims, in compliance with the revised amendment format, is shown above.

The amendments to the pending claims are made without prejudice, do not constitute amendments to overcome any prior art rejections under U.S.C. § 102, and are fully supported by the specification as filed. For example, support for the phrase "average optical density of stained target protein per pixel of cellular area" appears at, *inter alia*, page 11, line 26 to page 12, line 2. In addition, support for the cellular area being either the membrane or the nucleus appears in the same portion of the specification. Further support for all of the claim amendments can be found throughout the specification.

Cancellation of claims 15, 17, and 18 is without prejudice or disclaimer, and Applicant makes no admission regarding the patentability of this subject matter and should not be so construed. Applicant reserves the right to pursue this subject matter in this or in any other appropriate patent application.

The Office Action states that the previous Response to Office Action was filed by the Applicant on September 9, 2002. However, Applicant filed the response by Express Mail on September 3, 2002. Applicant has noted, however, that the correct filing date is recorded in the Patent Application Information Retrieval system, and understands that their previous response is considered timely filed by the Patent and Trademark Office.

In addition, the Office Action states that the Information Disclosure Statement filed on 9/9/02 failed to comply with 37 C.F.R. 1.97(c) because it lacks a statement as specified in 37 C.F.R. 1.97(e). However, such a statement is not required before the mailing of a final action, provided that the fee set forth in § 1.17(p) accompanies the response. Applicant authorized the Office to charge the deposit account for any fee, and therefore Applicant complied with 37 C.F.R. 1.97(c). Therefore, Applicant requests that these three references be considered at this time. A replacement PTO Form 1449 accompanies this response.

Discussion of the 35 U.S.C. § 112, ¶ 2 Rejection

Claims 2-13 and 15-20 are rejected under 35 U.S.C. § 112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant has overcome this ground of rejection in part and respectfully traversed in part.

With respect to claim 6, the Office Action stated that the claim is vague and indefinite in relation to claim 15 from which it then depended because it is unclear how the "immunological reagent" recited in the claim relates structurally and/or functionally to the "immunohistochemical stain: detectably labeled antibody" recited in claim 15. In addition, with respect to claim 15, subpart a), the Office Action stated that it fails to distinctly define how the quantity of target protein in the first portion of control cell pellets is "determined." In addition, in the same claim 15, subpart d), the Office Action states that it is unclear how a calibration curve is generated relating the target protein quantity obtained in a) with the average protein density of target protein obtained in c), in the absence of how the quantity of target protein in a) is determined. Applicant has cancelled claim 15 in favor of new claim 21, pursuant to her representatives'

comments in the informal interview with the Examiner to better clarify the claimed invention, thereby overcoming the Examiner's rejections. In addition, claim 6 is herein amended to clarify the relationship between claim 6 and new claim 21. Applicant respectfully contends that these amendments overcome the asserted ground of rejection.

In addition, the Office Action stated that claim 17 and claim 18 contain negative limitations that render these claims indefinite, because these claims appear to include elements/limitations not actually disclosed, thereby rendering the scope of the claims unascertainable. Although Applicant respectfully disagrees with the rejection of claim 17 and claim 18 and believes these claims are fully in compliance with § 112, nevertheless, in interests of expediting issuance of the remaining claims, Applicant has cancelled claim 17 and claim 18, thereby obviating rejection of these claims.

Applicant respectfully contends that the outstanding grounds of rejection of the pending claims under 35 U.S.C. § 112, ¶ 2 have been overcome by amendment in part, traversed by argument in part, or rendered moot by cancellation of the rejected claims. In view of the above, Applicant respectfully requests withdrawal of all 35 U.S.C. § 112, ¶ 2 rejections.

Discussion of the 35 U.S.C. § 112, ¶ 1 Rejection

Claims 17-19 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The rejection is respectfully traversed.

With respect to claim 19, the Office Action stated that the specification provides no literal or descriptive support for the recitation of "the calibration curve is linear." On the contrary,

however, there is support in the application as filed for this limitation. For example, on page 6, lines 13-16, the specification states "[t]he calibration curves produced according to and used with the methods of the invention are also advantageously expressed as an algorithm, most preferably in the form of a *linear* or logarithmic equation." (emphasis added). Thus, the specification provides literal support for the recitation of "the calibration curve is linear."

In addition, with respect to claim 17 and claim 18, the Office Action states that there is no specific guidance for the limitations present in these claims. Although Applicant respectfully disagrees with the rejection of claim 17 and claim 18 and believes these claims are fully in compliance with § 112, nevertheless, in the interest of expediting issuance of the remaining claims, Applicant has cancelled claim 17 and claim 18, thereby obviating rejection of these claims.

Applicant respectfully contends that the outstanding grounds of rejection of the pending claims under 35 U.S.C. § 112, ¶ 1 have been traversed by argument, or rendered moot by cancellation of the rejected claims. In view of the above, Applicant respectfully requests withdrawal of the 35 U.S.C. § 112, ¶ 1 rejection.

Discussion of the 35 U.S.C. § 102 Rejection

Claims 2-11, 13, 15-18, and 20 are rejected under 35 U.S.C. § 102(b) as being inherently anticipated by Slamon *et al* (U.S. Patent No. 5,846,749) ("Slamon"). Applicants respectfully traverse these rejections with the following arguments.

Under 35 U.S.C § 102, a claim is anticipated only if each and every element as set forth in the claim is found in a single art reference. M.P.E.P. § 2131; *Verdegall Bros. V. Union Oil Co.*, 814 F.2d 628, 631, 2 U.S.P.Q.2d (BNA) 1051, 1053 (Fed. Cir. 1987). The single prior art

reference must disclose the claimed invention identically and in as complete detail as is contained in the claim. M.P.E.P. § 2131; *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 U.S.P.Q.2d 1913, 1920 (Fed. Cir. 1989).

Here, the instantly claimed invention is directed towards methods for determining the quantity of a target protein in a biological sample. This method requires, among other things, that the average optical density of stained target protein per pixel of cellular area be determined by, for example, image analysis. Therefore, the actual number of cells present in the image field is irrelevant and never need be actually determined.

Slamon does not anticipate the present invention because it does not teach or suggest every element as set forth in the claims. Specifically, it does not teach a method of determining an "average optical density of stained target protein per pixel of cellular area." Instead, Slamon quantitates surface membrane and cytosolic proteins by determining the signal value from a *known* number of cells, and relates this value to values obtained with control cells. Thus, according to the Slamon reference the signal value *per cell* is determined, rather than the average optical density of stained target protein *per pixel* of cellular area. As an example, all of the independent method claims of the Slamon patent, require "determining the signal value *from a known number of said fixed cells* by computerized image analysis." *See, e.g., Slamon*, column 16, lines 12-14 (emphasis added). As a further example, the specification provides for measurement of protein immunostaining, specifically HER-2/*neu*, and measurement of DNA content in individual tumor cells. *See, e.g., Id.* at column 10, lines 52-57. The Slamon reference does not, however, teach how to quantitate cellular proteins using image analysis *without* knowing or determining the number of cells that are immunostained. Moreover, the Slamon reference does not teach how to determine the "average optical density of stained target protein

per pixel of cellular area," let alone how to use that information to determine the quantity of a target protein in cells of a biological sample. As a consequence, Slamon does not teach every limitation of the present invention.

In addition, the image analysis method disclosed in Slamon resulted in inherent problems for accurately and reliably quantitating target protein. For example, image analysis according to Slamon is accomplished by analyzing the immunostaining of the entire microscopic field, defined as an area of approximately 500 square microns. *Slamon* at column 6, lines 3-5. Tissue in this microscopic field will include the cells of interest, as well as additional cells and extracellular material. This constitution of the field was even noted in Slamon, stating that "stromal cells, connective tissue, lymphocytes, and normal tissue were not immunostained" in sample preparations, regardless of whether the sample was prepared from frozen tissue or paraffin-embedded material. *Id.* at column 13, lines 21-24. In order to compensate for this problem, Slamon disclosed analysis of 5 different fields, and suggested that usually 2-10 different fields need be analyzed with the results averaged together. *Id.* at column 5, line 67-column 6, line 3 and column 13, line 51-54. Although not explained in Slamon, this practice was presumably used to compensate for variability in staining observed when only one microscopic field was analyzed.

In contrast, the methods of the present invention do not contain the limitations present in the method of Slamon. As an example, instead of requiring compensation for "stromal cells, connective tissue, lymphocytes, and normal tissue" found in the microscopic field, as required by Slamon, the present invention includes no extraneous material from the field in the calculation. This is because the optical density of the extraneous material is not included in the "per pixel of cellular area" calculation. As explained in an example of the present inventive method, the

average optical density per pixel "corresponds to the total number of pixels comprising the membrane or nuclear area of a tissue sample," and therefore, correspondingly, the calculation does not include the optical density of pixels not falling within the cellular area of interest. *Application* at page 11, lines 30-31. This can be accomplished, for example, by immunohistochemically staining the target protein and counterstaining the tissue with another optical enhancement factor, such as ethyl green. *Id.* at page 8, lines 10-17 (noting that ethyl green is merely an exemplary, albeit useful, counterstain). This counterstain can be used to form a mask image of the tissues under consideration. *Id.* at page 9, lines 3-4. Then, the "average optical density of stained target protein per pixel of cellular area" is calculated "using only those areas of the image that are stained and which are within the mask." *Id.* at page 9, lines 4-9. Thus, any extraneous material, such as "stromal cells, connective tissue, lymphocytes, and normal tissue," is not considered in the calculation, thereby providing the present invention with an advantage over the Slamon method.

Applicants respectfully contend that rejection on 35 U.S.C. § 102 grounds has been traversed by their argument herein, and request that this rejection be withdrawn.

Discussion of the 35 U.S.C. § 103 Rejections

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over Slamon in view of McNamara et al. (U.S. Patent 6,007,996) ("McNamara"). In addition, claim 19 is rejected under 35 U.S.C. 103(a) as being patentable over Slamon. Applicants respectfully traverse this ground of rejection.

The instantly claimed invention is directed to a method for determining the quantity of a target protein in cells of a biological sample. As stated above, this method requires determining the average optical density of stained target protein per pixel of cellular area.

To establish a *prima facie* case of obviousness, the Office must identify one or a combination of references showing: (1) a teaching, suggestion, or motivation to practice the claimed invention, and (2) a reasonable expectation of success of achieving the invention. The teaching, suggestion or motivation to practice the claimed invention and the reasonable expectation of success must both be found in the prior art, and must not be based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991); M.P.E.P. § 2143.

None of the cited references, alone or in combination, teach or suggest the claimed invention because these claims require the average optical density of stained target protein per pixel of cellular area be determined as an affirmatively recited step of the claim, and neither reference teaches, suggests, or motivates the use of these cells for performing the claimed methods. The deficiencies of Slamon was discussed with regard to the rejection of claims 2-11, 13, 15-18, and 20 under 35 U.S.C. § 102. Simply stated, Slamon does not teach or suggest the claimed invention because it does not teach the determination of the average optical density of stained target protein per pixel of cellular area.

The deficiencies of Slamon are not overcome by its combination with McNamara. McNamara discloses, among other things, a method of *in situ* analysis of a biological sample comprising the steps of staining the biological sample with at least three stains, and collecting spectral data from the stained biological sample, where the spectral data device can collect data from all the stains. *See McNamara*, column 55, line 66-column 56, line 24. McNamara does not provide any teaching whatsoever related to determining the average optical density of stained

target protein per pixel of cellular area, and therefore cannot be combined with Slamon to arrive at the present invention.

Applicant respectfully contends that rejection on 35 U.S.C. § 103 grounds has been traversed by their argument herein, and request that this rejection be withdrawn.

Conclusion

In view of the above remarks, the application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone call would expedite the prosecution of this application, the Examiner is invited to call the undersigned attorney.

Respectfully Submitted,

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